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THE EFFECT OF TRAINING AND DETRAINING ON MUSCLE COMPOSITION IN THE HORSE

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SUMMARY

1. Percutaneous needle biopsies were obtained from six limb muscles in six horses before and during a training programme of 10 or 15 weeks designed to involve both aerobic and anaerobic work. In a subsequent detraining period, biopsies were also taken after 5 and 10 weeks.

2. Samples were analysed biochemically for enzyme activity of lactic dehydrogenase (LDH), creatine phosphokinase (CPK), aldolase (ALD), citrate synthase (CS), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) and for glycogen content. Fibre typing was carried out histochemically before and 10 weeks after commencement of training.

3. There was a significant increase in the percentage of high myosin ATPase activity pH 9·4/high oxidative (FTH) fibres with a corresponding decrease in high myosin ATPase activity pH 9·4/low oxidative (FT) fibres and low myosin ATPase activity pH 9·4/high oxidative (ST) fibres after 10 weeks training.

4. During training, enzyme activities increased progressively but at different rates with an approximate twofold increase in all of the enzymes except CPK by the end of the training period. Changes in all the muscles studied were similar. Glycogen content increased by approximately 33 % which was significant when all the muscles were considered together.

5. A decrease in enzyme activity occurred after 5 weeks detraining. However at 10 weeks a consistent but inexplicable increase in all enzyme levels, except CS again occurred.

6. It is concluded that training increased greatly the activity of enzymes involved in both aerobic and anaerobic metabolism.

INTRODUCTION

Over the past decade studies in several species have shown that with training there are alterations in skeletal muscle composition (Holloszy,

1973; Holloszy & Booth, 1976). Surprisingly few investigations have been carried out in the horse, a species which has been specifically developed for speed and heavy work. It has been reported that Standard bred racing horses of different ages have different muscle enzyme activities and this was attributed to a training effect (Lindholm & Piehl, 1974). Another study in horses has also shown that training results in an increase in succinic dehydrogenase (SDH) and malic dehydrogenase (MDH), and in the volume density of central mitochondria (Straub, Howald, Gerber, Diehl & Pauli, 1975). The purpose of this study was to examine alterations in enzyme activities and glycogen in a number of muscle groups in horses undergoing a training programme using a longitudinal study, i.e. sampling the same horses at different times.

METHODS

Six healthy horses consisting of four Thoroughbreds and two Heavy Hunters were studied (Table 1). These horses had undergone only maintenance exercise, consisting of walking and slow cantering, for the previous 3 months. Before training (0 weeks), muscle biopsies were taken from six limb muscles – *deltoideus*, long head of *triceps brachii*, *lateral vastus*, *middle gluteal*, *biceps femoris and semitendinosus* – with the percutaneous needle biopsy technique (Bergström, 1962), as described by Snow & Guy (1976). In order to allow specimens to be taken from all muscles, tranquillization was necessary in most animals.

TABLE 1. Physical characteristics of the horses at week 0

Horse no.	Breed	Age (yr)	Sex	Weight (kg)
1	Thoroughbred	9	Gelding	513
2	Thoroughbred	6	Gelding	437
3	Thoroughbred	5	Gelding	446
4	Thoroughbred	5	Mare	454
5	Heavy Hunter	12	Gelding	669
6	Heavy Hunter	13	Mare	656

The horses were put through a training programme which consisted of 4 days submaximal endurance work, the distance being gradually increased until the horses were trotting and cantering approximately 10–15 km per day, and 2 days maximal sprinting (galloping over 3 × 600 m) per week. This training programme lasted for 10 weeks. With the four Thoroughbreds a further period of 5 weeks sprinting over various distances was also included. At the end of the training period, the horses were slowly detrained with walking and slow cantering over 2 weeks, in order to prevent fluid accumulation in lower limbs, then only walking exercise (being led as opposed to be ridden) for 1 km per day during the rest of the detraining period.

Biopsies were taken from the six sites at weeks 0, 5, 10 and, in the case of the Thoroughbreds, week 15, and at 5 and 10 weeks during detraining. Before the biopsies were taken, the horses were given only walking exercise for 3 days in order to minimize any effects of acute exercise. The biopsy sites were all taken from within a 10 cm square and at a similar depth in order to minimize possible variation in the

muscle. The biopsies were divided into two at weeks 0 and 10, one piece being used for histochemistry, the other for biochemistry. At other times, only a specimen for biochemistry was collected.

The sample for histochemistry was orientated under a dissecting microscope in order to give transverse fibre sections when cut. The muscle was then placed on filter paper, covered in talcum powder to prevent ice artifacts forming and frozen in liquid nitrogen. Later this was mounted on a chuck with OCT embedding medium (Ames Tissue Tek), serial sections 10 μm thick cut in a cryostat at $-20°$ C and mounted on a glass slide. These sections were then used to estimate the activity of myosin adenosine triphosphatase pH 9·4 (ATPase) and succinate dehydrogenase (SDH) by the methods of Padykula & Herman (1955) and Nachlas, Walker & Seligman (1957) respectively. Muscle fibres were then typed.

The sample used for biochemistry was immediately frozen in liquid nitrogen and later weighed and then freeze dried (Speedivac Pearse, Tissue Dryer Mk I, Edwards High Vacuum) at $-40°$ C, 0·05 torr to remove all H_2O. After the weight was constant, the muscle was powdered in an agate mortar and pestle, and any connective tissue, fat or blood removed. Known amounts of powder were then homogenized in a solution of 150 mM-KCl, 50 mM-$KHCO_3$, 6 mM-EDTA and 1% horse serum albumin in distilled H_2O to give a 1 mg/ml. solution. Suitable dilutions of this homogenate were used to assay the following enzymes – lactic dehydrogenase (LDH) (E.C. 1.1.1.27), creatine phosphokinase (CPK) (E.C. 2.7.3.2), aldolase (ALD) (E.C. 4.1.2.7), citrate synthase (CS) (E.C. 4.1.3.7), aspartate aminotransferase (AST) (E.C. 2.6.1.1) and alanine aminotransferase (ALT) (E.C. 2.6.1.3). LDH, CPK, AST and ALT were assayed on an LKB 8600 Reaction Rate Analyzer using commercial kits (Boehringer nos. 15741, 15721, 19523 and 15924 respectively). ALD and CS were measured on a Pye Unicam SP 8000 U.V. spectrophotometer with the methods of Anderson (1975) and Srere (1969) respectively. All assays were carried out at 37° C. Glycogen was assayed according to the method of Huijing (1970) with the modification that the glucose produced was assayed with a commercial kit (Boehringer 15755).

The precision of the assay methods were determined using the formula $S = \sqrt{(d^2/2N)}$, where d is the difference between duplicates of the same sample and N is the number of samples. The precision was for LDH 4·5%, CPK 6%, ALD 6·0%, CS 4·5%, AST 3%, ALT 3·5% and glycogen 2·1%. Within-batch variation was LDH 3·9%, CPK 5·4%, ALD 3·5%, C.S. 5·7%, AST 6%, ALT 4·3% and glycogen 3·9%, and between batch variation was LDH 4·6%, CPK 6·9%, ALD 7·4%, C.S. 6·7%, AST 6·9%, ALT 11% and glycogen 7·3%.

Significant differences for each parameter measured at different times were tested for using a 'paired' t test. Correlations between the percentage fibre type and the enzyme activities at weeks 0 and 10 were also determined.

RESULTS

Three fibre types were found in horse skeletal limb muscles on the basis of histochemical staining. They were classified as high myosin ATPase activity (pH 9·4)/low oxidative, high myosin ATPase activity (pH 9·4)/high oxidative and low myosin, ATPase activity (pH 9·4)/high oxidative. Work by Bárány (1967) and Guth & Samaha (1969) has shown that high myosin ATPase activity at pH 9·4 is indicative of a fast contractile speed of the fibre and although no direct evidence is available in the horse this

assumption has been made by some workers (Davies & Gunn, 1972; Lindholm & Piehl, 1974). In the interest of consistency, we have used the abbreviations of Lindholm & Piehl (1974) (FT, FTH and ST respectively for the above three fibre types) in preference to one of the many other similar classifications in use (Ashmore & Doerr, 1971; Barnard, Edgerton, Furukawa & Peter, 1971; Peter, Barnard, Edgerton, Gillespie & Stempel, 1972) with the proviso that high myosin ATPase activity at pH 9·4 is only probably indicative of a fast contracting fibre.

TABLE 2. Percentage fibre types (mean ± S.E. of mean) before and after 10 weeks training for six limb muscles (n = numbers in parentheses)

	ST		FTH		FT	
	Before	After	Before	After	Before	After
Deltoid	35·3 ± 4·1 (6)	28·4 ± 3·9* (5)	27·7 ± 4·1 (6)	41·9 ± 5·0* (5)	37·0 ± 3·9 (6)	29·6 ± 3·4* (5)
Long head triceps	20·4 ± 2·4 (6)	19·3 ± 2·4 (5)	40·9 ± 1·7 (6)	46·3 ± 4·4 (5)	38·7 ± 2·1 (6)	34·3 ± 3·2 (5)
Lateral vastus	11·5 ± 1·9 (6)	6·9 ± 2·5 (4)	46·5 ± 3·2 (6)	50·6 ± 3·6 (4)	42·0 ± 2·5 (6)	38·8 ± 1·2 (4)
Middle gluteal	15·5 ± 3·1 (6)	17·5 ± 1·8 (6)	50·0 ± 2·2 (6)	48·8 ± 1·4 (6)	34·5 ± 2·0 (6)	33·7 ± 1·3 (6)
Biceps femoris	19·6 ± 2·0 (6)	16·9 ± 2·4 (4)	43·0 ± 2·9 (6)	49·7 ± 4·5 (4)	37·4 ± 1·7 (6)	33·4 ± 1·2 (4)
Semitendinosus	14·0 ± 2·7 (6)	12·6 ± 3·3 (5)	55·0 ± 2·7 (6)	58·1 ± 5·7 (5)	31·0 ± 2·5 (6)	29·2 ± 3·8 (5)
Mean	20·0 ± 1·9 (36)	17·3 ± 1·6* (29)	44·9 ± 2·1 (36)	49·4 ± 1·8* (29)	35·1 ± 1·3 (36)	33·3 ± 1·2* (29)

* $P < 0·05$.

Mean values for the percentage fibre types before and after training are given in Table 2. The horses in this study were found to have a high percentage of high myosin ATPase (pH 9·4) fibres (FT and FTH) with the two Heavy Hunters having a lower percentage for most muscles.

From the limited number of subjects available, it appears that the front limb muscles, the deltoideus and possibly the long head of the triceps brachii have a higher percentage of ST fibres than the other muscles. The percentage of high oxidative fibres to low oxidative fibres is almost the same for all muscles studied.

With training, only the deltoideus muscle showed a significant change in fibre type, with a decrease in low myosin ATPase (pH 9·4) fibres and an increase in high oxidative fibres. Due to various reasons, some of the values for the fibre percentages are not available after training and the

TABLE 3. Effect of training and detraining on citrate synthase activity (μmole/min.g dry weight tissue) for six limb muscles. (mean ± S.E. of mean $n = 6$ (week 15, $n = 4$))

	Training				Detraining	
Muscle	0 weeks	5 weeks	10 weeks	15 weeks	5 weeks†	10 weeks
Deltoid	9·62 ± 0·76	12·58 ± 1·03	15·54 ± 1·77***	19·83 ± 1·48***	16·83 ± 2·56	21·83 ± 2·95
Long head triceps	13·82 ± 0·97	20·33 ± 1·01***	21·30 ± 1·46***	24·95 ± 1·25***	21·00 ± 2·49	22·75 ± 2·48
Lateral vastus	8·02 ± 1·17	9·00 ± 1·20	10·74 ± 0·94***	18·00 ± 3·43**	11·25 ± 2·46	13·50 ± 2·29
Middle gluteal	15·84 ± 1·12	17·11 ± 2·45	21·88 ± 2·34**	28·62 ± 1·52***	16·78 ± 3·56***	25·25 ± 3·72
Biceps femoris	16·36 ± 1·64	19·55 ± 2·55	22·29 ± 2·86**	30·88 ± 4·25***	24·42 ± 2·88	26·20 ± 3·80
Semitendinosus	12·92 ± 1·82	18·30 ± 2·45	18·95 ± 1·77**	27·00 ± 1·68***	19·91 ± 2·78**	17·08 ± 1·67
Mean	12·76 ± 0·71	16·15 ± 1·18***	19·42 ± 1·02**	25·08 ± 1·36***	18·64 ± 1·22**	20·95 ± 1·32

* $P < 0·05$.
** $P < 0·01$.
*** $P < 0·001$ compared with week 0.
† Significance symbols at 5 weeks detrained are when these levels are compared to last week of training.

TABLE 4. Effect of training and detraining on aldolase activity (μmole × 10^{-1}/min.g dry weight tissue) for six limb muscles (mean ± S.E. of mean, $n = 6$ (week 15, $n = 4$))

	Training				Detraining	
Muscle	0 weeks	5 weeks	10 weeks	15 weeks	5 weeks†	10 weeks
Deltoid	18·38 ± 2·12	25·43 ± 1·83***	30·52 ± 4·43**	27·83 ± 1·92	28·88 ± 3·63	38·05 ± 3·50
Long head triceps	24·00 ± 2·25	34·43 ± 3·23***	40·00 ± 2·25***	38·40 ± 5·33*	33·90 ± 2·35*	40·00 ± 2·15
Lateral vastus	24·86 ± 3·14	33·24 ± 3·09	40·48 ± 2·16**	42·78 ± 4·35*	35·37 ± 2·23*	43·65 ± 2·57
Middle gluteal	31·82 ± 2·25	41·02 ± 2·33**	46·18 ± 2·28***	47·50 ± 3·00**	41·14 ± 1·88*	49·70 ± 2·20
Biceps femoris	24·92 ± 1·90	33·60 ± 2·15*	38·97 ± 2·82**	40·10 ± 2·05***	34·93 ± 1·43	41·80 ± 4·49
Semitendinosus	31·05 ± 2·57	40·87 ± 2·62	45·17 ± 3·08***	49·30 ± 2·52***	40·78 ± 2·27	50·40 ± 1·65
Mean	25·50 ± 1·20	34·70 ± 1·33***	40·20 ± 1·41*	41·50 ± 1·20***	35·80 ± 1·15**	44·00 ± 1·35

* $P < 0·05$.
** $P < 0·01$.
*** $P < 0·001$ compared with week 0.
† Significance symbols at 5 weeks detrained are when these levels are compared to last week of training.

TABLE 5. Effect of training and detraining on lactic dehydrogenase activity (μmole $\times 10^{-2}$/min.g dry weight tissue) for six limb muscles (mean ± S.E. of mean, $n = 6$ (week 15, $n = 4$))

	Training				Detraining	
Muscle	0 weeks	5 weeks	10 weeks	15 weeks	5 weeks†	10 weeks
Deltoid	10·93 ± 1·39	17·38 ± 1·47***	19·57 ± 2·15*	20·39 ± 2·17*	19·56 ± 2·42	24·15 ± 2·54
Long head triceps	15·04 ± 2·00	23·13 ± 3·04**	25·06 ± 2·13***	22·53 ± 2·23*	23·24 ± 2·69	27·95 ± 2·51
Lateral vastus	17·50 ± 0·81	24·47 ± 2·78	28·54 ± 2·94***	33·47 ± 3·70**	27·27 ± 2·42	30·02 ± 2·38
Middle gluteal	23·93 ± 2·07	33·73 ± 1·90***	27·55 ± 1·71**	37·84 ± 2·84***	33·82 ± 1·93	39·13 ± 1·71
Biceps femoris	17·27 ± 1·18	24·98 ± 2·13*	27·52 ± 1·71**	30·74 ± 0·45***	28·30 ± 1·25	29·84 ± 1·59
Semitendinosus	21·48 ± 2·84	32·68 ± 2·91	31·99 ± 1·59***	34·88 ± 1·15***	34·09 ± 1·69	37·80 ± 2·85
Mean	17·69 ± 0·99	26·05 ± 1·32***	28·74 ± 1·48***	30·39 ± 1·57***	27·73 ± 1·19*	31·63 ± 1·24

* $P < 0·05$.
** $P < 0·01$.
*** $P < 0·001$ compared with week 0.
† Significance symbols at 5 weeks detrained are when these levels are compared to last week of training.

TABLE 6. Effect of training and detraining on creatine phosphokinase activity (μmole $\times 10^{-3}$/min.g dry weight tissue) for six limb muscles (mean ± S.E. of mean $n = 6$ (week 15, $n = 4$))

	Training				Detraining	
Muscle	0 weeks	5 weeks	10 weeks	15 weeks	5 weeks†	10 weeks
Deltoid	14·39 ± 0·96	16·66 ± 0·82	19·53 ± 0·95***	20·63 ± 0·31	19·48 ± 1·66	23·64 ± 1·61
Long head triceps	16·21 ± 1·04	18·75 ± 1·48	21·57 ± 1·13***	21·20 ± 0·24	18·97 ± 1·35	22·75 ± 1·49
Lateral vastus	15·68 ± 0·90	17·11 ± 1·07	20·66 ± 1·55*	22·58 ± 1·08*	18·98 ± 0·85	21·99 ± 1·84
Middle gluteal	16·95 ± 0·75	17·90 ± 1·34	21·34 ± 1·36**	23·17 ± 0·88***	19·88 ± 0·81	23·82 ± 1·73
Biceps femoris	16·77 ± 0·87	18·73 ± 0·83	21·28 ± 2·06	23·60 ± 1·03***	20·97 ± 0·88	23·28 ± 2·12
Semitendinosus	14·86 ± 0·54	16·58 ± 1·23	20·26 ± 0·70***	22·99 ± 0·84***	20·34 ± 1·16	24·81 ± 0·99
Mean	15·79 ± 0·36	17·63 ± 0·46***	20·78 ± 0·53***	22·43 ± 0·37***	19·77 ± 0·45***	22·78 ± 0·65

* $P < 0·05$.
** $P < 0·01$.
*** $P < 0·001$ compared with week 0.
† Significance symbols at 5 weeks detrained are when these levels are compared to last week of training.

TABLE 7. Effect of training and detraining on alanine aminotransferase activity (μmole/min.g dry weight tissue) for six limb muscles (mean ± S.E. of mean $n = 6$ (week 15, $n = 4$))

	Trained				Detrained	
Muscle	0 weeks	5 weeks	10 weeks	15 weeks	5 weeks†	10 weeks
Deltoid	31·30 ± 3·45	28·98 ± 3·79	46·28 ± 5·52*	61·00 ± 2·94*	61·15 ± 10·97	73·25 ± 13·17
Long head triceps	30·07 ± 4·94	47·13 ± 8·04**	61·15 ± 4·60***	64·90 ± 3·93***	69·00 ± 9·98	78·20 ± 15·30
Lateral vastus	27·30 ± 4·87	28·96 ± 4·06	38·88 ± 6·93	62·55 ± 7·91*	38·06 ± 6·04	51·53 ± 10·89
Middle gluteal	33·48 ± 4·99	37·78 ± 7·15	65·70 ± 8·10***	92·37 ± 11·15*	56·11 ± 9·36	76·18 ± 15·63
Biceps femoris	33·45 ± 4·50	44·60 ± 8·00	63·88 ± 9·84**	90·67 ± 8·08**	70·18 ± 10·73	77·05 ± 19·73
Semitendinosus	33·90 ± 4·45	40·00 ± 5·00	53·95 ± 4·51*	86·90 ± 8·76*	62·33 ± 11·97	65·40 ± 8·21
Mean	31·50 ± 1·70	37·80 ± 2·60**	54·90 ± 3·00***	77·00 ± 4·00***	57·40 ± 4·10***	70·20 ± 5·60

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$ compared with week 0.
† Significance symbols at 5 weeks detrained are when these levels compared to last week of training.

TABLE 8. Effect of training and detraining on aspartate aminotransferase activity (μmole × 10^{-1}/min.g dry weight of tissue) for six limb muscles (mean ± S.E. of mean $n = 6$ (week 15, $n = 4$))

	Trained				Untrained	
Muscle	0 weeks	5 weeks	10 weeks	15 weeks	5 weeks†	10 weeks
Deltoid	36·35 ± 5·00	52·03 ± 7·66*	70·15 ± 5·02***	87·27 ± 5·34***	71·77 ± 11·30	92·60 ± 12·14
Long head triceps	44·23 ± 5·77	75·58 ± 12·82**	98·48 ± 7·86***	104·63 ± 5·31**	76·62 ± 11·44	96·47 ± 12·04
Lateral vastus	35·12 ± 6·07	45·28 ± 7·57	55·03 ± 8·53**	78·17 ± 6·30**	53·13 ± 11·79	62·90 ± 12·12
Middle gluteal	51·73 ± 6·97	67·03 ± 1·52	78·52 ± 6·18	122·15 ± 9·35***	77·18 ± 11·87***	103·72 ± 15·67
Biceps femoris	52·98 ± 9·79	76·10 ± 12·62	69·28 ± 7·89	114·15 ± 12·19**	96·08 ± 10·74	104·95 ± 17·39
Semitendinosus	43·66 ± 5·66	74·10 ± 10·52*	68·45 ± 5·12	119·32 ± 9·34***	91·71 ± 15·52	88·15 ± 9·69
Mean	44·00 ± 2·70	65·00 ± 4·70***	73·30 ± 3·40***	105·00 ± 4·60***	74·90 ± 5·30***	91·40 ± 5·60

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$ compared with week 0.
† Significance symbols at 5 weeks detrained are when these levels compared to last week of training.

resulting small number of subjects may be the reason that more muscle groups do not show a significant change. However, when all the muscles were considered together a significant decrease in low myosin ATPase (pH 9·4) fibres and a significant increase in high oxidative fibres were also found with training. This had the effect of increasing the percentage of FTH fibres at the expense of both the FT and ST fibres.

Enzyme activities for the Thoroughbreds and Heavy Hunters were considered together as levels and changes were similar in the two breeds. Tables 3–8 show the mean values for the enzyme activities of the six

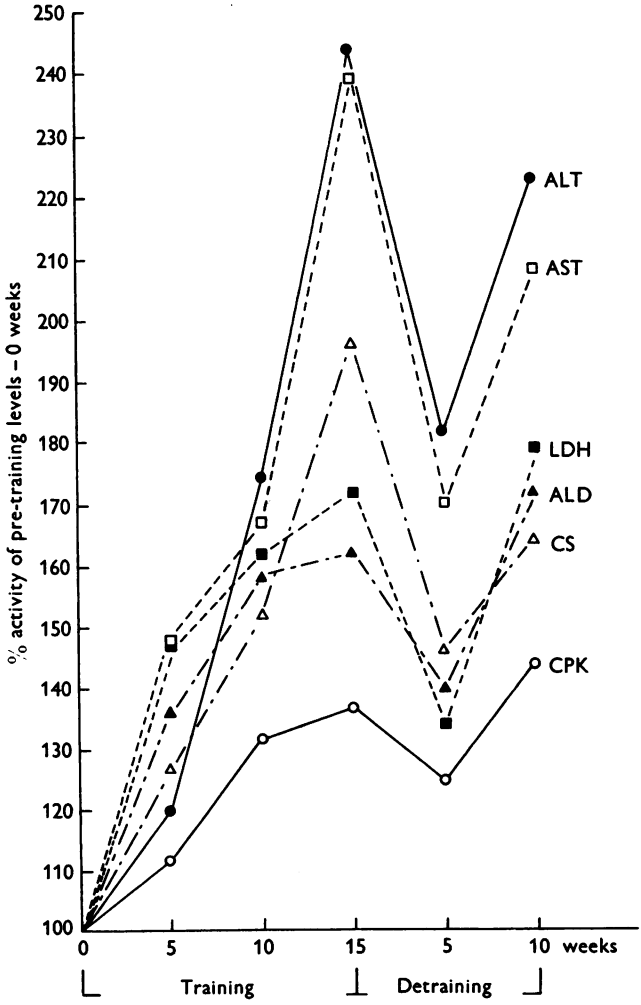

Fig. 1. Percentage activity of pre-training levels (mean of all muscles) *vs.* time for several enzymes in horse skeletal muscle.

horses for the limb muscles investigated. Because of the small number of animals, the mean activity of each enzyme for all muscles at each period of sampling was also calculated to determine whether there was an over-all change in activities for each enzyme with training. As can be seen from the tables, training resulted in an increase of approximately 70–100% for all the enzymes studied with the exception of CPK which increased approximately 30%. As these changes are much larger than the errors in our methods they must be considered as changes due to training. As can be

TABLE 9. Effect of 10 weeks training on the correlation between percentage high myosin ATPase fibres and enzyme activity

		r	Level of significance
Lactic dehydrogenase	0 weeks	0·43	$P<0·01$
	10 weeks	0·34	n.s.
Creatine phosphokinase	0 weeks	0·29	n.s.
	10 weeks	0·23	n.s.
Aldolase	0 weeks	0·39	$P<0·02$
	10 weeks	0·27	n.s.
Citrate synthase	0 weeks	0·09	n.s.
	10 weeks	0·12	n.s.
Aspartate aminotransferase	0 weeks	0·26	n.s.
	10 weeks	0·00	n.s.
Alanine aminotransferase	0 weeks	0·27	n.s.
	10 weeks	0·16	n.s.

n at 0 weeks = 36, at 10 weeks = 29.

seen from Fig. 1, the increases in the enzyme levels occurred at different rates. It is interesting to note that the greatest increase in the anaerobic metabolism enzymes LDH and ALD was in the first 5 weeks whereas that of CPK and ALT occurred during the second 5 weeks. The percentage change in AST and CS was similar for both periods of time. In the case of the four Thoroughbreds, the further 5 weeks of sprint training perhaps surprisingly produced no great increase in activity of the anaerobic enzymes ALD, LDH and CPK whereas the activity of ALT, AST and CS all increased by more than in either of the two previous 5-week periods.

A linear relationship was found between the percentage of total fast twitch fibres (FTH and FT) and the glycolytic enzymes LDH and ALD for all the muscles in all the animals at week 0 (Table 9). This correlation was not evident at week 10. None of the other enzymes showed a significant correlation at either period of sampling.

After 5 weeks detraining all of the mean enzyme activities had decreased significantly over the corresponding levels at the end of the training

TABLE 10. Effect of training and detraining on glycogen concentration (μmole/g dry weight tissue) for six limb muscles (mean $\pm$ S.E. of mean, $n = 6$ (week 15, $n = 4$))

	Trained				Untrained	
Muscle	0 weeks	5 weeks	10 weeks	15 weeks	5 weeks†	10 weeks
Deltoid	265 ± 19·8	326 ± 18·8	271 ± 27·7	350 ± 5·8	297 ± 32·9	320 ± 22·7
Long head triceps	297 ± 15·4	376 ± 12·7	360 ± 303	444 ± 23·2*	408 ± 31·4	347 ± 15·4
Lateral vastus	280 ± 18·4	294 ± 17·2	278 ± 17·2	322 ± 9·9	289 ± 21·1	276 ± 27·5
Middle gluteal	348 ± 27·8	367 ± 40·6	374 ± 27·7	459 ± 6·6	399 ± 27·7	429 ± 16·4
Biceps femoris	299 ± 13·4	328 ± 22·3	374 ± 23·2	448 ± 28·9**	397 ± 22·7	436 ± 16·4
Semitendinosus	323 ± 25·4	340 ± 23·0	376 ± 25·5	479 ± 5·5*	406 ± 26·2	351 ± 20·1
Mean	302 ± 9·0	338 ± 11·3**	339 ± 12·4*	420 ± 14·0***	366 ± 13·5	361 ± 12·1

* $P < 0{\cdot}05$.
** $P < 0{\cdot}01$.
*** $P < 0{\cdot}001$ compared with week 0.
† Significance symbols at 5 weeks detrained are when these levels compared to last week of training.

period (10 weeks for Heavy Hunters and 15 weeks for Thoroughbreds) but were still significantly higher than 0-week levels. However, surprisingly, following 10 weeks detraining, an opposite effect to that seen at 5 weeks resulted. A significant increase in mean activities of each enzyme except citrate synthase occurred at the 10 week stage over that determined at 5 weeks detraining.

The increase in glycogen content following training (Table 10) was found to be significant at each stage of training when the results from all the muscles were considered. During detraining glycogen levels remained elevated over those at 0 weeks.

DISCUSSION

Horse skeletal muscle, like that of most mammals, is composed of a mixed population of fibres distributed in a mosaic pattern. The finding of three types in the Thoroughbred and Heavy Hunter is in agreement with those found in the Standardbred by Lindholm & Piehl (1974).

Reports on the effect of training on fibre type have been contradictory and largely depend on whether the oxidative or myosin ATPase activity of the fibres are considered. Morgan, Cobb, Short, Ross & Gunn (1971), staining only for SDH an oxidative enzyme, concluded that there was a change in fibre type with training. However, in studies where both oxidative and myosin ATPase properties were examined, it is generally agreed that whilst an increase in the staining for oxidative enzymes may occur, no change in the proportion of high to low myosin ATPase activity of the fibres occurs (Barnard, Edgerton & Peter, 1970; Gollnick, Armstrong Saltin, Saubert IV, Sembrowich & Shepherd, 1973; Lindholm & Piehl, 1974). The results in this study appear to agree that an increase in high oxidative fibres occurs but, surprisingly, we have also found a decrease in the percentage of low myosin ATPase fibres. Although a change in the percentage of high myosin ATPase to low myosin ATPase fibres has not been reported in studies using training as the physiological stimulus, studies on rabbits have shown that chronic electrical stimulation with implanted electrodes induces a change from a fast to a slow contractile speed with a parallel change in the percentage of high and low myosin ATPase (Brown, Cotter, Hudlicka & Vrbova, 1976; Pette, Muller, Leisner & Vrbova, 1976). Similarly, Staute, Exner & Pette (1973) have found a decrease in the twitch contraction time in the soleus muscle of rats which have undergone high intensity sprint training and concluded that here is an increase in the proportion of fast oxidative fibres with high intensity training. It is therefore not inconceivable that in the horse an increase in FTH fibres would occur at the expense of both FT and ST fibres.

All the enzymes studied in this investigation increased to varying

degrees with training. Similar studies in various species have produced differing results even within the same species. These differences may arise according to whether aerobic and/or anaerobic metabolism was primarily involved in the training programme. The extent to which the enzyme activities change will also depend on the training programme as the percentage change in enzyme activity is proportional to the intensity of exercise (Fitts, Booth, Winder & Holloszy, 1975). As similar changes in activity of a given enzyme occurred in each muscle in our horses, it appears that all of the limb muscles studied were being worked to a similar degree of activity.

The approximately twofold increase in citrate synthase activity following training confirms the greater oxidative capacity seen with histochemical staining. This change probably occurs due to the endurance exercise part of the training programme as varying increases with endurance training in the tricarboxylic acid (TCA) cycle enzymes, succinic dehydrogenase and citrate synthase have been reported in man (Gollnick *et al.* 1973), rat (Holloszy, Oscai, Don and Molé, 1970), guinea-pig (Barnard *et al.* 1970), and pig (Fogd Jorgensen & Hyldgaard-Jensen, 1975). In association with this increase in TCA cycle enzymes, there is also an increase in both the size and number of skeletal muscle mitochondria (Gollnick, Ianuzzo & King, 1971), and a change in the composition of the mitochondria (Holloszy, Molé, Baldwin & Terjung, 1973). Although this increase in respiratory capacity of the myofibres occurs with training no increase in the level of O_2 consumption results with a given submaximal exercise (Holloszy, 1973). Therefore in the trained individual, the O_2 uptake per mitochondrion is lower, with less of a decrease in the concentrations of creatine phosphate and ATP per mitochondrion. As ATP is an allosteric inhibitor of glycolysis (Hofmann, 1976) this adaptation will result in a decreased rate of glycolysis for any given work load, and will be responsible for a glycogen sparing action. Glycogen depletion has been indicated to be a causative factor in the onset of muscle fatigue (Ahlborg, Bergström, Ekelund & Hultman, 1967), therefore this glycogen sparing effect following training will increase the duration to exhaustion with submaximal exercise. This effect was apparent in our horses as they all appeared less fatigued following a standard endurance run after training than before.

In addition to the above mechanism causing a decreased utilization of glycogen with training, another contributing factor to glycogen sparing is the increased ability of muscle to metabolize fat as an energy source (Issekutz, Miller & Rodahl, 1966). This adaptation occurs due to an increase in enzymes involved in free fatty acid degradation (Molé, Oscai & Holloszy, 1971) as well as the increase in activity of TCA cycle enzymes.

Increased oxidation of fat will lead to increased citrate levels which have been shown to inhibit phosphofructokinase, a limiting factor in glycolysis (Hofmann, 1976).

As well as the above adaptations in aerobic metabolism occurring with endurance exercise adaptations in anaerobic metabolism to allow increased maximal work loads may also occur. This present study indicates that an increase in glycolytic activity occurred as indicated by the twofold increase in aldolase. It is assumed that this change was caused by the anaerobic exercise, as other studies (Gollnick *et al.* 1973; Lindholm & Piehl, 1974) have reported that training involving high anaerobic requirements cause increases in glycolytic activity, whilst studies on endurance training have generally demonstrated little change in these enzymes (Baldwin, Winder, Terjung & Holloszy, 1973; Fogd Jorgensen & Hyldgaard-Jensen, 1975).

The large elevations in LDH activity with training seen in this study are at variance with the changes reported in other species, where little change (Fogd Jorgensen & Hyldgaard-Jensen, 1975) or even a decrease (Baldwin, *et al.* 1973; Suominen & Heikkinen, 1975), has been found. However, most of these investigations have been involved with only endurance training and as already described this results in a decrease in the rate of glycogen break-down and consequently pyruvate production. This results in a decrease in the requirement for anaerobic degradation of pyruvate. It has also been reported in man (Hermansen, Hultman & Saltin, 1967) that following training the same relative work load results in a decreased production of lactate which again indicates a decreased requirement for LDH. On the other hand, in these horses an increase in lactate production was found after training for a given sprint exercise involving being ridden flat out for 600 m (Snow & MacKenzie, 1977). However, although no direct evidence was available to show that the horses were being exercised at the same relative work load, it is thought that the increase in lactate production and LDH activity are linked. Why this adaptive difference between man and horse occurs is not known. It may be that during sprinting the requirements for glycolysis are greater in the horse due to a greater energy demand which cannot be met by the increased metabolism of fat. As already described training results in an increased glycolytic capacity and if increased glycolysis occurs increased levels of pyruvate will result. The pyruvate will be unable to enter the TCA cycle due to the increased metabolism of fat producing acetyl CoA which inhibits the pyruvate dehydrogenase complex (Randle, Garland, Hales, Newsholme, Denton & Pogson, 1966). Therefore increased LDH activity will result in more rapid conversion of pyruvate to lactate and the replenishment of NAD^+ to permit further glycolysis.

The linear relationship found between the percentage total high myosin ATPase fibres (FT and FTH) and both the glycolytic enzymes examined is to be expected as high myosin ATPase fibres have a greater glycolytic activity than low myosin ATPase fibres in the horse (P. S. Guy & D. H. Snow, unpublished). The results for LDH also agree with those found in man (Karlsson, Frith, Sjodin, Gollnick & Saltin, 1974). It has to be noted however, that these correlations although significant are not all that large. The non-significance of these correlations after training may therefore be due to the decreased number of muscles available.

A third pathway for pyruvate metabolism is the conversion to alanine by ALT and increased levels of this metabolite have been reported following exercise in man (Felig & Wahren, 1971; Bloom, Johnson, Park, Rennie & Sulaiman, 1976), and the horse (D. H. Snow and R. J. Chalmers, unpublished data). With training increases in this enzyme have been demonstrated in rats (Molé, Baldwin, Terjung & Holloszy, 1973) and in this present study. An increase in ALT would be advantageous especially during anaerobic exercise as it would permit greater amounts of pyruvate to be metabolized to alanine. This would aid in maintaining intracellular homoeostasis by reducing lactate production and hence the decrease in pH. High lactate and low pH have been implicated as a cause of fatigue with strenuous exercise (Karlsson & Saltin, 1970) by causing inhibition of glycolysis at the phosphofructokinase level (Hofmann, 1976) and also by interfering with muscular contractions due to the H^+ ions competing with Ca^{2+} for the binding sites that control actin–myosin interactions (Katz, 1970). The increase in ALT takes place after that of LDH and it may be speculated that the increase in ALT occurs to counteract the increase in lactate that may arise from this earlier adaptation.

CPK is another enzyme which is connected with sprinting as high intensity exercise makes large demands on the mechanisms which supply immediate energy in the cell. These mechanisms are, in the main, replenishment of ATP from creatine phosphate by CPK and ADP by myokinase. Therefore, an increase in CPK activity with training would increase ATP resynthesis for high intensity sprinting. The increase of 30% in CPK activity found in this study was much lower than for the other enzymes studied but is similar to that found in man (Thorstensson, Sjodin & Karlsson, 1975), and rat (Staudte *et al.* 1973), using sprint training. Other studies using endurance training have found no change in man (Souminen & Heikkinen, 1975), pig (Fogd Jorgensen & Hyldgaard-Jensen, 1975) and rat (Oscai & Holloszy, 1971).

As extramitochondrial NADH produced during glycolysis is unable to penetrate mitochondria for oxidation, a number of indirect routes, called shuttles, have been defined and are thought to allow electrons derived

from NADH to enter the electron transport chain. The best documented of these shuttles are the α-glycerophosphate shuttle and the malate–aspartate shuttle (Van Dam & Meyer, 1971). Studies in the rat have shown that with training there is an increase in the enzymes of the malate–aspartate shuttle (Holloszy, Booth, Winder & Fitts, 1975) whilst no change occurred in the α-glycerophosphate shuttle (Holloszy & Oscai, 1969). Our present studies also indicate an increase in the aspartate shuttle, as there is a twofold increase in AST. In conjunction with the increase on ALT this increase in AST will aid in the maintenance of cellular pH during marked glycolytic activity.

The high values of glycogen in this study are similar to those reported by Lindholm & Piehl (1974) in the horse and are greater than those reported for most other species (Rülcker, 1968; Gollnick *et al.* 1973; Fitts *et al.* 1975). Our increase in glycogen concentration in skeletal muscle with training is in agreement with that observed in man (Gollnick *et al.* 1973), rat (Fitts *et al.* 1975) and pig (Rülcker, 1968), although the changes are of a smaller magnitude than in most species. The already high levels of glycogen in the horse may explain the lower increases, as increases in glycogen synthetase activity have been reported to be involved in the mechanism for increased glycogen content (Piehl, Adolfsson & Nazar, 1974) and perhaps the high levels of glycogen are indirectly inhibiting this enzyme activity (Huijing, Nuttall, Villar-Palasi & Larner, 1969).

A significant decrease was found in all of the enzyme activities of the combined muscles after 5 weeks detraining. Several studies have been reported on the effect of detraining on heart weight:bodyweight ratio (Secher, 1921; Steinhaus, Kirmiz & Lauritsen, 1932) and regression of adrenal and heart weight and liver cholesterol after training (Gollnick & Simmons, 1967), however, there appears only one study of the histochemical changes in guinea-pig skeletal muscle where detraining caused a regression of red fibres compared to white fibres (Faulkner, Maxwell & Liebermann, 1972) which suggests a return of the muscle characteristics to those before training. Further confirmation of this is found in a study of rats where a lower level of work was given to the rats after a period of training and the levels of SDH decreased correspondingly (Benzi, Panceri, Bernardi, Villa, Arcelli, d'Angelo, Arrigoni & Berté, 1975).

The 'rebound' effect occurring after 10 weeks detraining is surprising, as it would have been expected that the levels of enzyme activity would have decreased further towards the level at week 0. No satisfactory explanation of this effect can at present be given and no comparable studies found in the literature. It can only be speculated that the effect has been caused by the abnormal sedentary mode of life of the animals. It has indeed been found in man (Bass, Vondra, Rath, Vitek, Teisinger, Mackova,

Sprynorova & Malkovaska, 1975) that sedentary subjects, i.e. subjects who were receiving little or no exercise, had an abnormal pattern of enzyme activities in the lateral vastus when compared to trained subjects and competition athletes, and also when compared to other mammals. It may therefore be that the increased activities of the enzymes in the horse after 10 weeks detraining is the start of an unusual enzyme pattern.

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